Rapid and Efficient Generation of Functional Motor Neurons From Human Pluripotent Stem Cells Using Gene Delivered Transcription Factor Codes.

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Public Summary:

Stem cell-derived motor neurons (MNs) are utilized for modeling disease in vitro and for developing cellular replacement strategies for spinal cord injury and diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS or Lou Gehrig's Disease). Human embryonic stem cell (hESC) differentiation into MNs, which involves the use o a number of neurotrophic factors is inefficient and requires up to 60 days to develop MNs with electrophysiological properties. This prolonged differentiation process has hampered the use of hESCs, in particular for high-throughput screening. We evaluated the MN gene expression profile of hESCs during differntiation to MNs to identify rate-limiting factors involved in MN development. Based on this analysis, we developed a viral gene delivery system encoding for 3 factors that were highly expressed in MN (neurogenin 2, islet-1, and LIM/homeobox protein 3). Strikingly, delivery of these factors induced functional MNs with mature electrophysiological properties, 11-days after gene delivery, with >60-70% efficiency from hESCs and human induced pluripotent stem cells (hiPSCs). This directed programming approach significantly reduces the time required to generate electrophysiologically-active MNs by approximately 30 days in comparison to conventional differentiation techniques. The results described in this publication further exemplify the potential to use viral vectors to deliver factors that will promote rapid and efficient production of defined cell types from hESCs and hiPSCs.

Scientific Abstract:

Stem cell-derived motor neurons (MNs) are increasingly utilized for modeling disease in vitro and for developing cellular replacement strategies for spinal cord injury and diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Human embryonic stem cell (hESC) differentiation into MNs, which involves retinoic acid (RA) and activation of the sonic hedgehog (SHH) pathway is inefficient and requires up to 60 days to develop MNs with electrophysiological properties. This prolonged differentiation process has hampered the use of hESCs, in particular for high-throughput screening. We evaluated the MN gene expression profile of RA/SHH-differentiated hESCs to identify rate-limiting factors involved in MN development. Based on this analysis, we developed an adenoviral gene delivery system encoding for MN inducing transcription factors: neurogenin 2 (Ngn2), islet-1 (Isl-1), and LIM/homeobox protein 3 (Lhx3). Strikingly, delivery of these factors induced functional MNs with mature electrophysiological properties, 11-days after gene delivery, with >60-70% efficiency from hESCs and human induced pluripotent stem cells (hiPSCs). This directed programming approach significantly reduces the time required to generate electrophysiologically-active MNs by approximately 30 days in comparison to conventional differentiation techniques. Our results further exemplify the potential to use transcriptional coding for rapid and efficient production of defined cell types from hESCs and hiPSCs.